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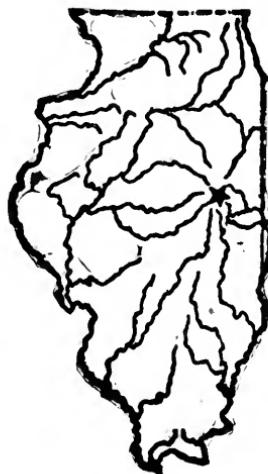
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UNIVERSITY OF ILLINOIS
Agricultural Experiment Station

BULLETIN No. 190

SOIL BACTERIA AND PHOSPHATES

BY CYRIL G. HOPKINS AND ALBERT L. WHITING



URBANA, ILLINOIS, JUNE, 1916



FOREWORD

The fundamental facts of soil fertility and crop production are slowly but surely becoming established, and the farmer has begun to shape his agricultural practice by the discoveries of science. Of these discoveries the following may fairly be called fundamental.

The fact established by Sénébier of Switzerland that the carbon of crops is derived from the atmosphere and not from the soil.

The work of DeSaussure which clearly demonstrated that mineral elements are essential to plant growth.

The work of Lawes and Gilbert showing that farm crops are unable to take the nitrogen from the atmosphere thru the leaf.

The experiments of Atwater and Woods establishing the fact that leguminous crops are in some way able to utilize atmospheric nitrogen.

The experiments of Hellriegel establishing the fact that the principal agents in the fixation of atmospheric nitrogen are certain bacteria growing upon the roots of leguminous plants.

Investigations at the University of Illinois concerning the amounts of nitrogen which may be brought into the soil by means of these bacteria, under field conditions.¹

Later experiments by the University of Illinois concerning the availability of natural sources of mineral plant food in permanent agriculture.¹

The purpose of the experiment herein reported was to determine whether the farmer supplying nitrogen to his soil in the form of organic matter could depend upon its decomposition to render soluble the finely ground rock phosphate, and so maintain the necessary supply of available phosphorus. This probability has long been suspected by the University, and the results reported seem to indicate conclusively that in the presence of liberal applications of finely ground rock phosphate such solution may be depended upon. This discovery ranks in importance with the inoculation of legumes and with the determination of the amount of atmospheric nitrogen that can be fixed by leguminous crops.

When we remember that chemistry as a science dates practically from the time of the American Revolution, when King George was more interested in better agriculture for England than in quieting his turbulent colonies, and when we recall that the great science of bacteriology has entirely developed since the time of our Civil War and the abolition of slavery, it is evident that rapid progress is being made in the establishment of farming on a truly scientific basis, and that slow as discovery seems to be, it is after all relatively rapid.

E. DAVENPORT, *Director.*

¹See Ill. Agr. Exp. Sta. Buls. 76 (Alfalfa on Illinois Soil), 94 (Nitrogen Bacteria and Legumes), 182 (Potassium from the Soil).

SUMMARY OF BULLETIN No. 190

1. Nitrite bacteria make phosphorus and calcium soluble from insoluble phosphates when they oxidize or convert ammonia into nitrite. Page 401
2. The actual ratio found shows that about one pound of phosphorus and about two pounds of calcium are made soluble for each pound of nitrogen oxidized, aside from the action of the acid radicles associated with the ammonia. Page 402
3. The ratio of solubility found on the basis of nitrogen to phosphorus and calcium conforms to the following reaction:
$$4\text{HNO}_2 + \text{Ca}_3(\text{PO}_4)_2 \rightleftharpoons \text{CaH}_4(\text{PO}_4)_2 + 2\text{Ca}(\text{NO}_2)_2$$
According to this equation, 56 pounds of nitrogen liberate in soluble form 62 pounds of phosphorus and 120 pounds of calcium. Page 403
4. Plants are important factors in the liberation of phosphorus, owing to the production of carbon dioxid and the removal of the soluble phosphorus produced by the bacteria. Page 404
5. Neither ammonia-producing bacteria nor nitrate bacteria liberate appreciable amounts of soluble phosphorus from insoluble phosphates. Pages 398, 403
6. Other acid-producing bacteria make phosphorus soluble from insoluble phosphates according to the nature and amount of the acid produced. Pages 403, 405
7. A comparison of the amounts of nitrogen, phosphorus, and calcium required by farm crops, with those possible of solution by biochemical action, shows possibilities far beyond the plant requirements; which leads to the conclusion that plenty of rock phosphate in contact with decaying organic matter must give the plants an excellent opportunity to obtain both phosphorus and calcium as well as nitrogen. Page 405

SOIL BACTERIA AND PHOSPHATES

BY CYRIL G. HOPKINS, CHIEF IN AGRONOMY AND CHEMISTRY, AND
ALBERT L. WHITING, ASSOCIATE IN SOIL BIOLOGY

Raw rock phosphate is by far the cheapest source of phosphorus to apply to soils. It consists chiefly of tricalcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$, which is the most common form of phosphorus in the great natural deposits. This phosphorus compound is relatively insoluble in water, and, for this reason, it has been argued by some that it does not become available to plants; but long-continued field experiments, pot-culture experiments, and farm practice have fully demonstrated that this kind of phosphate does become available for plant growth. (See Circulars 181 and 186).

With the results obtained in practice confirming on a large scale the experimental results, it became a laboratory problem to determine how this insoluble phosphate is made soluble by changes occurring in soils. The increased beneficial results obtained by following the practice commonly recommended of intimately mixing decaying organic matter with the phosphate lead to the suggestion that the action of the soil bacteria that decompose organic matter might be an important factor in the solution of the phosphate. The investigation reported in this bulletin has proved that this hypothesis was correct, for these bacteria have been found capable of making the phosphate soluble. The data hereinafter recorded show that the action is even more direct than we had reason to believe. It has been the common teaching that nitrifying bacteria require the presence of a free base, such as lime or an alkaline carbonate, but we have found that the bacterial action produces acid phosphate and proceeds in the presence of this acid salt.

DECOMPOSITION OF ORGANIC MATTER BY SOIL BACTERIA

The organic matter of soils consists of the remains of plant roots, stems, and leaves, and of farm manures. These are made up of proteins, sugars, starches, fiber, and other less important compounds, such as fats. All these constituents are subject to decay by bacteria. They are broken down by bacteria into less complex substances. This breaking down is not brought about by one kind of bacteria only, nor is it the result of but one process.

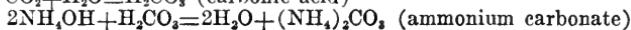
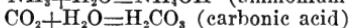
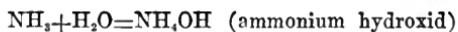
The importance of the action of decomposition products of the active organic matter¹ of the soil on the solubility of phosphates is better understood by a consideration of three important and definitely

¹Organic matter which is capable of rapid decomposition is called active organic matter, and that which is resistant to decay is known as humus or inactive organic matter.

recognized processes that have long been known to bring about the change of the nitrogen from the unavailable form, as it occurs in the protein of clover, manure, etc., to the readily available form of the nitrate, as found in calcium nitrate ("nitrate of lime"), sodium nitrate ("nitrate of soda"), potassium nitrate ("saltpeter"), etc.

There are three well known steps, or stages, in the biochemical process of converting organic nitrogen into nitrate nitrogen:

(1) **Ammonia Production.**—The first process results in the change of the organic nitrogen to ammonia nitrogen. The ammonia (NH_3) is absorbed by the soil moisture (H_2O) and forms ammonium hydroxid (NH_4OH), or household ammonia. Much carbon dioxid (CO_2) is produced at the same time, and some of it, also, is absorbed by the soil moisture and then unites with the ammonia to form ammonium carbonate. The formation of this ammonium carbonate is represented by the following equations:¹



Ammonia, ammonium hydroxid, and ammonium carbonate are alkaline. Red litmus is turned blue by these compounds. This should be carefully noted, as only acids are able to make phosphates and limestones soluble, as explained elsewhere.

If more carbon dioxid is produced than will unite with the ammonia produced, it will dissolve in the soil moisture, forming carbonic acid, and it may make raw rock phosphate soluble, as will be shown later. The soil moisture, however, will hold only a certain amount of this gas and any above that amount is returned to the air, which is the source of carbon for plants.

While the ammonia is being produced, other compounds are likewise being formed, and such organic acids as the acetic acid of vinegar,

¹These equations are easily understood by reference to the following table of atomic weights and valences, hydrogen being taken as the standard, or unit, of measure:

Element	Symbol	Atomic weight	Valence
Hydrogen	H	1	1
Oxygen	O	16	2
Nitrogen	N	14	3 or 5
Carbon	C	12	4
Phosphorus	P	31	3 or 5
Calcium	Ca	40	2
Sulfur	S	32	2

The symbol O stands for an atom of oxygen with a weight of 16 and a valence of 2. The term *valence* means the number of bonds of attraction possessed by an atom. These bonds may be considered as hands or hooks. Thus, the two-handed oxygen atom may hold two one-handed hydrogen atoms in the compound called water, H_2O , or $\text{H}-\text{O}-\text{H}$. While the elements hydrogen and oxygen are both gases, H_2O is the formula of a molecule of the liquid compound, water, whose molecular weight is 18, the sum of the atomic weights.

$2\text{Ca}_3(\text{PO}_4)_2$ is read, "two Ca three, PO four twice," and means two molecules of tricalcium phosphate, each consisting of three atoms of calcium and twice the parenthetic group containing one atom of phosphorus and four of oxygen. One molecule weighs 310 (times the hydrogen atom), as readily computed from the atomic weights.

the lactic acid of sour milk, the butyric acid of rancid butter, and many others result. These will unite with bases, such as lime, or will react with and decompose such mineral compounds as raw rock phosphate and ground limestone. In this way they will dissolve, or liberate, phosphorus and calcium.

The potato and the hay bacteria,¹ which are very common in all soils, are vigorous producers of ammonia. Many other kinds of soil bacteria decompose organic matter into ammonia, carbon dioxid, and organic acids. These ammonifying bacteria, however, are unable to convert ammonia into any other compounds. By their action ammonia is always being produced in soils. It will be produced rapidly or slowly according to the conditions which prevail in a given portion of the soil. The production of ammonia in soils seldom approaches that observed in manure piles or composts. Small amounts of ammonia are always present in soils in a good state of fertility, but nitrate, which is derived from ammonia, is normally present in larger amounts. The presence of nitrate demonstrates that a change from an alkaline condition to an acid condition is always taking place, for to produce nitrate requires nitrous or nitric acid. Even when manure and raw rock phosphate are composted, nitrate forms in large amounts, altho a test of the mass as a whole shows it to be alkaline: at many local points acid nitrogen must be formed, otherwise nitrate production would be impossible.

As ammonia is the most important compound obtained in the first stage of the decomposition of organic matter, this stage is called ammonification or ammonia production.

(2) **Nitrite Production.**—The second and most important of the three stages consists of the oxidation of the ammonia to nitrite. In order to form a nitrite, nitrous acid (HNO_2) must be produced. This nitrous acid is very similar to nitric acid (HNO_3). The oxidation of ammonia to nitrous acid by the nitrite bacteria is represented by the following equation:



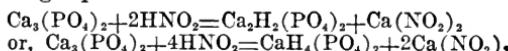
The ammonium portion of the ammonium carbonate, an alkaline compound, has been converted into nitrous acid and carbonic acid has been set free. Both these acids will combine with some base. It is important to note that nitrogen of the alkaline substance, ammonia, has been converted, or transformed, by the biochemical removal of hydrogen and addition of oxygen into a strongly acid substance, nitrous acid.

This strong acid must be neutralized by some base, if nitrification is to proceed, for the bacteria are inactive in the presence of any noticeable amounts of strong acid. If the acid is neutralized, a ni-

¹The potato bacteria (*Bacillus mesentericus*) are so called on account of their being easily found in the eyes of potatoes; the so-called hay bacteria (*Bacillus subtilis*) are found very abundantly in hay and at one time were thought to cause hay fever.

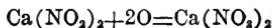
trite is the product formed, such as calcium nitrite, $\text{Ca}(\text{NO}_2)_2$. The primary purpose of this investigation is expressed in the question, Will the calcium of pure rock phosphate, $\text{Ca}_3(\text{PO}_4)_2$, suffice as a base; and if so, will the phosphorus be made soluble? This will be answered by the experimental data reported in another part of this bulletin.

If nitrite production takes place with tricalcium phosphate as a source of the base calcium, then the reaction must be represented by one of the following equations:



The bacteria which oxidize the ammonia to nitrite are called nitrite bacteria, or *Nitrosomonas*, and only one kind is known which is able to perform this intermediate step in the transformation of organic nitrogen to nitrate. These bacteria are unable to use any other than ammonium compounds.

(3) **Nitrate Production.**—The third and last stage is a simple oxidation of the nitrite to nitrate by the action of nitrate bacteria (*Nitrobacter*). It consists in the addition, by biochemical action, of oxygen to the nitrite:



This reaction increases neither acidity nor alkalinity, but it produces nitrate, a compound of nitrogen which is preferred by nearly all forms of plant life. No liberation of insoluble compounds would be expected in this process, as no additional base is necessary, as seen by reference to the equation.

These nitrate bacteria live only on nitrite, and consequently they must await the action of the nitrite bacteria. While it is known that nitrites are formed in soils under field conditions, only ammonia and nitrate can be found, as the nitrate bacteria change the nitrite as rapidly as it is formed.

INFLUENCE OF AMMONIA PRODUCTION ON SOLUBILITY OF PHOSPHATES

As already stated, the most important product formed in the first process, or stage, of the decomposition of the organic matter is ammonium carbonate. The ammonium carbonate is alkaline, and consequently could not be expected to exert any action on the solubility of raw rock phosphate.

In 1904 Stalstrom of Finland conducted laboratory experiments on the solubility of pure rock phosphate with bacteria which produced ammonium carbonate from peat and from manure containing peat litter. He concluded that there was no appreciable increase in solubility of phosphorus where the bacteria had produced ammonium carbonate over the sterile treatments in which no ammonium carbonate

was produced. His experiments lasted forty-two days and were under conditions which would permit of determining soluble phosphorus were it present. His work is extremely interesting as it demonstrates that in the first stage of decomposition it has been impossible to measure any soluble phosphorus without the growing plant as an indicator.

Similar results have been obtained by the Rhode Island and Wisconsin Experiment Stations in attempts to detect soluble phosphorus in fermenting mixtures of manure and raw rock phosphate and in mixtures of soil and raw rock phosphate. When, however, plants have been grown in the mixtures, crop yields have demonstrated an advantage from the intimate contact of the phosphate with the decaying organic matter. Even when soluble phosphates are applied to similar mixtures, it soon becomes difficult to find soluble phosphorus owing to the alkaline condition of the mass as a whole. However, this does not preclude the possibility of phosphorus having been made soluble at many local points, strong evidence of which is afforded by the growing plants.

SOLUTION OF PHOSPHATES BY ACTION OF NITRITE BACTERIA

Until the work reported in this bulletin was undertaken, the part played by the nitrite bacteria in dissolving mineral compounds, and particularly raw rock phosphate, had never been investigated, and to determine this was our principal object in these experiments.

One of the authors made the following suggestion several years ago:

"In the conversion of sufficient organic nitrogen into nitrate nitrogen for a hundred-bushel crop of corn, the nitric acid, if formed, would be alone sufficient to convert seven times as much insoluble tricalcium phosphate into soluble monocalcium¹ phosphate as would be required to supply the phosphorus for the same crop."²

The following equation shows that nitrous acid makes raw rock phosphate soluble.



Expressed in other terms: 188 pounds of nitrous acid mixed with 310 pounds of pure rock phosphate make 234 pounds of acid phosphate and 264 pounds of calcium nitrite, both of which are soluble. Thus, 56 pounds of nitrogen, when oxidized to nitrous acid, have power to dissolve 62 pounds of phosphorus and 120 pounds of calcium, contained in rock phosphate. If the nitrite bacteria bring about the reaction shown by the equation, both calcium and phosphorus are thus dissolved from raw rock phosphate. The bacteria need the calcium to neutralize the nitrous acid produced, and a small amount of both calcium and phosphorus is needed for their own bodies.

¹*Mono-* means one; *di-*, two; *tri-*, three; *tetra-*, four; *penta-*, five. Mono-calcium phosphate, which may also be called monocalcium tetrahydrogen phosphate, is an acid phosphate, the acidity being due to the hydrogen.

²Hopkins' "Soil Fertility and Permanent Agriculture," page 197.

EXPERIMENT I: NITRITE BACTERIA AND PURE ROCK PHOSPHATE

The purpose of this experiment was to test the ability of nitrite bacteria to dissolve pure rock phosphate. The methods of determining their ability and its extent consisted in actually measuring by chemical analysis the amount of nitrogen which they had changed from ammonia to nitrite and the amount of phosphorus and calcium which had at the same time been made soluble.

The plan of the experiment, briefly stated, was as follows: A thin layer (about $\frac{1}{8}$ inch thick) of a nutrient salt solution was placed in a cone-shaped glass flask of about one quart capacity and about 5 inches in diameter at the bottom. In this solution was placed a definite amount of the insoluble pure tricalcium phosphate and a definite amount of ammonium salt. The flasks and materials were sterilized to kill all bacteria and molds. Nitrite bacteria isolated from corn-belt soil were then introduced from pure cultures or directly from the soil. Cotton plugs were kept in the mouths of the flasks to prevent the entrance of other bacteria. The flasks were kept at a temperature of 82° Fahrenheit. Many such flasks were prepared, and later, usually at intervals of one week, the contents of two or more flasks were analyzed for nitrogen changed or oxidized and for water-soluble phosphorus and calcium.¹

¹The details of the experiment are given, as follows, for those desiring more information:

The flasks were one-liter Erlenmeyers, and 25 cc. of the salt solution was added to each one. The salt solution contained, per liter, 1 gram sodium chlorid, 250 milligrams potassium sulfate, 100 milligrams magnesium sulfate, and 3 drops ferric chlorid.

The flasks containing the nutrient solution were plugged with cotton and sterilized in the autoclave. The salt solution, tricalcium phosphate, and nitrogen solutions were analyzed before being used and were sterilized before being added to the flasks. These chemicals were especially prepared to free them from impurities. To each flask was added 45 milligrams tricalcium phosphate and 10.585 milligrams nitrogen as ammonium sulfate. In some cases the nitrogen was increased to 21.17, 42.34, and 84.68 milligrams per flask to test the effect of concentration. Pure cultures of nitrite bacteria were used in some experiments. These had been isolated on silica-jelly from typical corn-belt soil. In some experiments the bacteria were added directly from the soil in 5 cc. of an infusion made of 2 parts water and 1 part soil. The soil infusion was allowed to settle, the supernatant liquid was then drawn off into a beaker and further settling allowed before the infusion was added to the flasks. This prevented the addition of soil particles, which might furnish a free base. The soil infusion appears more satisfactory than pure cultures.

The colonies grew on the surface of the liquid, forming a bluish mass, some developing to $\frac{1}{4}$ inch in diameter. Typical colonies of this kind were isolated from the impure soil infusion cultures on silica-jelly plates. Colonies $\frac{1}{8}$ inch in diameter developed. They were colorless to opalescent at first and later a glassy blue, center showing yellow after fourteen days and later orange yellow to brown. When stained with gentian violet, they appeared as typical *Nitrosomonas*. Visible growth in solution was slow for the first forty days, but after that time a very profuse surface growth developed showing large blue colonies some of which were drawn up the sides of the flask by the surface tension of the liquid and

In Table 1 is shown the relative amounts, by weight, of nitrogen from ammonium sulfate oxidized to nitrite by nitrite bacteria and the amounts of phosphorus and calcium made soluble. Each figure represents the average of duplicate determinations.

TABLE 1.—NITROGEN OXIDIZED, AND PHOSPHORUS AND CALCIUM MADE SOLUBLE BY NITRITE BACTERIA¹
(Expressed in milligrams)

Flask No.	Duration in days	Nitrogen oxidized	Phosphorus made soluble	Calcium made soluble
1	28	2.54	4.08	3.87
2	41	3.81	5.08	5.60
3	41	...	5.99	8.40
4	48	5.52	9.56	14.80
5	48	4.88	10.20	18.40
6	55	6.40	12.85	22.00
7	55	6.40	10.24	23.52
8	62	6.88	16.00	31.04
9	48	3.61	7.52	13.60
10	62	3.87	8.76	16.48
11	62	5.84	9.82	16.00
12	62	5.68	11.28	20.80
13	69	6.03	11.14	22.40
14 ²	48	5.76	13.04	24.80
15 ²	69	4.60	11.60	19.20
16	139	18.84	41.56	75.26

¹The results show that the rate of solution varies even under conditions made as nearly alike as possible. Factors tending to produce these variations may include variations in the number of bacteria originally introduced and in the diffusion of carbon dioxid and oxygen thru the cotton plugs.

²Ammonium nitrate was used in Flasks 14 and 15 in place of ammonium sulfate.

EXPLANATION OF RESULTS

The results reported in Table 1 demonstrate conclusively that phosphorus and calcium are made soluble while the nitrite bacteria oxidize ammonia nitrogen to nitrite nitrogen. It is also evident that the solubility increases with increasing time of action of the bacteria.

there developed to a large size ($\frac{1}{4}$ inch in diameter). Five cubic centimeters sterile water was added every week to make up for evaporation. When the determinations were made, duplicate flasks were filtered thru the same filter (S. and S. blue ribbon 589) and washed with 25 cc. nitrogen-free cold distilled water. Sometimes double filters were used. The whole was made up to 200 cc. and the nitrogen oxidized determined by the Devarda method, phosphorus by the volumetric method, and calcium by the permanganate method.

A factor which may account for deviations in the results was the slime growth, which by absorption or by clogging of the filter possibly prevented the filtration and washing out of all the soluble calcium and phosphorus. Sterile checks were included to test the solubility of the tricalcium phosphate in pure water, in the salt solution, in the presence of the soil infusion, and in the presence of the ammonium salts, singly and in combination. The soluble contents of the flasks developed an acid reaction, as required by the formation of acid phosphate in the solution.

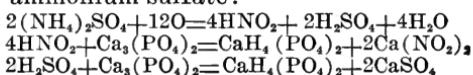
Ten cubic centimeters of Flask 16 required 3.35 cc. of N/12.5 NaOH with phenolphthalein as the indicator for the second hydrogen atom. The normality of the solution was found to be N/37.2. A soil with 20 percent moisture would require 535.9 pounds of calcium carbonate per acre to neutralize the acid produced by the bacteria.

An inspection of the figures shows that there is, by weight, approximately twice as much phosphorus and four times as much calcium made soluble as there is nitrogen oxidized by the bacteria. As an average of the results from thirteen flasks (Nos. 4 to 16), we find that from the oxidation of 56 pounds of nitrogen 115 pounds of phosphorus and 211 pounds of calcium are made soluble. The results from Flasks 1, 2, and 3 are not included in the ratio calculated or discussed, as there appears to be a utilization by the bacteria themselves of the calcium first liberated, the bacteria possibly storing it up in their bodies in order to have a reserve supply. (Thru an error, the nitrogen oxidized in Flask 3 was not secured.)

For a proper understanding of the results, an explanation of the ratio of oxidized nitrogen to soluble phosphorus and soluble calcium is essential.

Ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$, is made up of two chemical opposites, the ammonium (NH_4) being alkaline and the sulfate (SO_4) being an acid radicle. When they unite, two ammonium groups are required to neutralize one sulfate radicle.

When the nitrite bacteria oxidize the ammonium groups (NH_4) to the acid radicle (NO_2), an equivalent amount of sulfate radicle (SO_4) is free to act, with the nitrous acid formed, on the raw rock phosphate. As much calcium is required to combine with the sulfate as with the nitrite. All other substances which might combine with the acid formed or freed are already held in neutral combination. Thus, the nitrogen oxidized unites with one-half the calcium made soluble, while the sulfate radicle (SO_4), or the nitrate radicle (NO_3) (Flasks 14 and 15), unites with the other half. The equations below show this fact for ammonium sulfate:



According to these equations, when 56 pounds of nitrogen are changed from the ammonia form to the nitrite form, with both the nitrous acid (HNO_2) and the associated sulfuric acid (H_2SO_4) acting on the pure rock phosphate, 124 pounds of phosphorus and 240 pounds of calcium are made soluble and thus occurs the double action obtained on the insoluble phosphate in the results reported. The average results from the thirteen duplicate trials show that 115 parts of phosphorus (instead of 124) and 211 parts of calcium (instead of 240) were actually found in solution, and, in individual cases (Flasks 5, 10, 14, 8, 9, 16), the experimental results approached even more closely to the theoretical amounts. The results obtained with the ammonium nitrate are in agreement with those obtained with ammonium sulfate.

Interpreted in terms of farm practice, these results mean that every pound of organic nitrogen in manure or clover residues which is converted into nitrate may make slightly more than one pound of

phosphorus and two pounds of calcium soluble from raw rock phosphate, assuming of course an intimate contact of phosphate and decaying organic matter and that all the nitrous acid acts on the insoluble phosphate. The exact proportions are 56 of nitrogen, 62 of phosphorus, and 120 of calcium, as is readily computed from the reaction between four molecules of nitrous acid and one of tricalcium phosphate.

When insoluble tricalcium phosphate (pure rock phosphate) is converted into soluble monocalcium phosphate, the solution is made acid on account of the increased number of hydrogen atoms in solution. The soluble contents of the flasks used in this experiment should, therefore, be acid. The filtrates were tested with blue litmus paper and found to be acid. Some of the solutions, especially that in Flask 8, which represented complete solution of the phosphate, instantly turned blue litmus red.

The bacteria, after dissolving the phosphate, by subsequent action on the solution make some of the phosphorus insoluble again, possibly by utilizing it for their own growth. This actually occurred when the filtrates were allowed to stand a long time, unless sterilized. It is therefore important to make determinations periodically, as a reversion of the phosphate or a precipitation of calcium sulfate from a concentration of the solution by evaporation of the water, may obscure the true ratios.

EXPERIMENT II: NITRATE BACTERIA AND PURE ROCK PHOSPHATE

The results of an experiment to test the effect of the nitrate bacteria on pure tricalcium phosphate are seen by reference to Table 2. While all the nitrogen in one case and nearly all in the other had been oxidized to nitrate, practically no soluble phosphorus or calcium was found above that in the check; which fact of course supports the theory that no solution of phosphate is to be expected by the action of nitrate bacteria.

TABLE 2.—NITROGEN OXIDIZED FROM SODIUM NITRITE TO NITRATE, AND PHOSPHORUS AND CALCIUM MADE SOLUBLE BY NITRATE BACTERIA
(Expressed in milligrams)

Flask No.	Duration in days	Nitrogen oxidized	Phosphorus soluble	Calcium soluble
1	41	21.45	.00	.09
2	62	18.67	.00	.00

SOLUBILITY OF PHOSPHATES BY CARBON DIOXID¹

Carbon dioxid (CO_2) is produced in large amounts in soils by bacterial action on proteins, sugars, starches, fiber, and other compounds.

¹Carbon dioxid is a gas, but when dissolved in water, it forms carbonic acid, or carbonated water.

It is exhaled by bacteria in respiration just as it is by man. It has been found that one pound of non-symbiotic nitrogen-fixing bacteria (*Azotobacter*) exhaled 1.27 pounds of carbon dioxid in 24 hours. A man weighing 150 pounds and doing hard muscular work exhales only 3.74 pounds of carbon dioxid in 24 hours.

The enormous production of carbon dioxid by soil bacteria is due to their feeding upon easily oxidizable carbon and to their rapid multiplication. It is well known that manure and crop residues added to the soil greatly increase the carbon dioxid produced, as these materials are good food for bacteria. In one case a normal application of manure increased the carbon dioxid produced 33 percent. Very little carbon is retained by the bacteria compared to that given off.

The carbon dioxid produced by bacteria is absorbed in the soil moisture and when in contact with raw rock phosphate will dissolve it according to the following equation:



According to this, 176 pounds of carbon dioxid make 62 pounds of phosphorus soluble.

It is a simple matter to show the solubility of raw rock phosphate in water saturated with carbon dioxid. This was accomplished as early as 1868, by Knop. However, when soil is treated with carbonated water for long periods, only relatively small amounts of the phosphorus present can be found in a soluble form; and when bacteria which produce carbon dioxid in large amounts are allowed to act in soils, not much soluble phosphorus is produced unless large applications of carbonaceous material have been made for the bacteria to feed upon. When this is done, a more appreciable solubility is found.

Conditions are seldom favorable for more than a small portion of the carbon dioxid produced in the soil to act in the liberation of phosphorus from raw rock phosphate. Much of the carbon dioxid is dissipated in other ways and the greater portion returns to the air, it being a very volatile compound. This is evident from the work of Kröber, who found under very suitable conditions that it required the production of 122 pounds of carbon dioxid to liberate one pound of soluble phosphorus from pure rock phosphate.

It has long been known that plants excrete carbon dioxid from their roots. Kossovitsch found that the roots of mustard plants produced large amounts of carbon dioxid during a growing season. Stocklaza studied the production of carbon dioxid from wheat and clover roots and found that the daily production was much greater with the clover than with the wheat.

The effectiveness of carbon dioxid excreted by plant roots depends upon the number of plants per acre, the kind of plant, and the kind and amount of phosphate applied. The larger the application the greater the contact with the plant roots. It has been demonstrated by investigations with pot cultures at the University of Illinois that

common farm crops possess some ability to utilize rock phosphate without organic matter and that their ability to do this is increased by increased applications of the rock phosphate. It has also been shown that cereals possess this ability as well as legumes.

Rain water is another source of carbon dioxid, becoming charged with the gas from the air, in which there are four parts in 10,000; and such water possesses power to dissolve minerals, such as rock phosphate. That carbon dioxid is an important solvent of minerals is well understood.

SOLUBILITY OF PHOSPHATES BY ORGANIC ACIDS

The acid of vinegar (acetic), of rancid butter (butyric), of sour milk (lactic), and many other similar acids result from bacterial and mold action on carbohydrates, proteins, and fats in the soil. These acids may act on phosphates and render them soluble. They are much stronger in their action than carbonic acid and weaker than nitrous or nitric acid. They are produced in very small amounts compared with the production of carbon dioxid, but perhaps in larger amounts than nitrous acid. The percentage of phosphorus made soluble by these acids is probably higher under similar conditions than that made soluble by carbonic acid.

Sackett, Patten, and Brown of the Michigan Agricultural Experiment Station, and Koch and Kröber of Germany have shown that soil bacteria which produce organic acids make large amounts of bone meal and raw rock phosphate soluble. The addition of limestone with the insoluble phosphates prevents the detection of soluble phosphates. The undesirable condition created by the intimate contact of ground-limestone and rock phosphate in the soil is largely avoided by the methods of application recommended by the Illinois Station.

IMPORTANCE AND EXTENT OF THE ACTION OF NITRITE BACTERIA

It has already been shown that the nitrite bacteria make phosphorus and calcium soluble from pure rock phosphate and that the action conforms to a definite chemical ratio.¹

The nitrous acid² produced may act upon compounds of iron, aluminum, potassium, sodium, or magnesium which occur in soils, or it may act upon tricalcium phosphate, calcium silicate, or calcium carbonate, if present. For this reason, it has been recommended that the ideal practice to obtain the greatest solubility of the raw rock phosphate is to turn it under in intimate contact with organic matter, and,

¹It was found that the action of the nitrite bacteria was the same on the natural raw rock phosphate as on the pure rock phosphate, but more extensive experiments with the natural rocks will be reported later.

²It is not necessary to assume that the nitrous acid produced by the bacteria accumulates in the soil to a noticeable extent.

if needed, to apply ground limestone after plowing or at some other point in the crop rotation.

The rôle the plant plays in this process of utilizing natural plant food materials is highly important: it acts as a pump, removing from the soil the soluble phosphorus, calcium, and nitrogen, as they are formed, thus giving room for more soluble phosphorus, calcium, and nitrogen to be formed. Under these conditions the bacteria are stimulated to put forth their best efforts. When the soluble nitrogen, phosphorus, and calcium accumulate, they probably tend to reduce the activity of the nitrite bacteria, because bacteria do not thrive, as a rule, in an excess of their own product, but fortunately their action never ceases while suitable conditions exist.

In Table 3 are presented the actual amounts of phosphorus, calcium, and nitrogen required by standard crops, and the amounts of phosphorus and calcium which would be made soluble if all the nitrogen required for the crop should be oxidized to nitrate and should act upon pure rock phosphate.

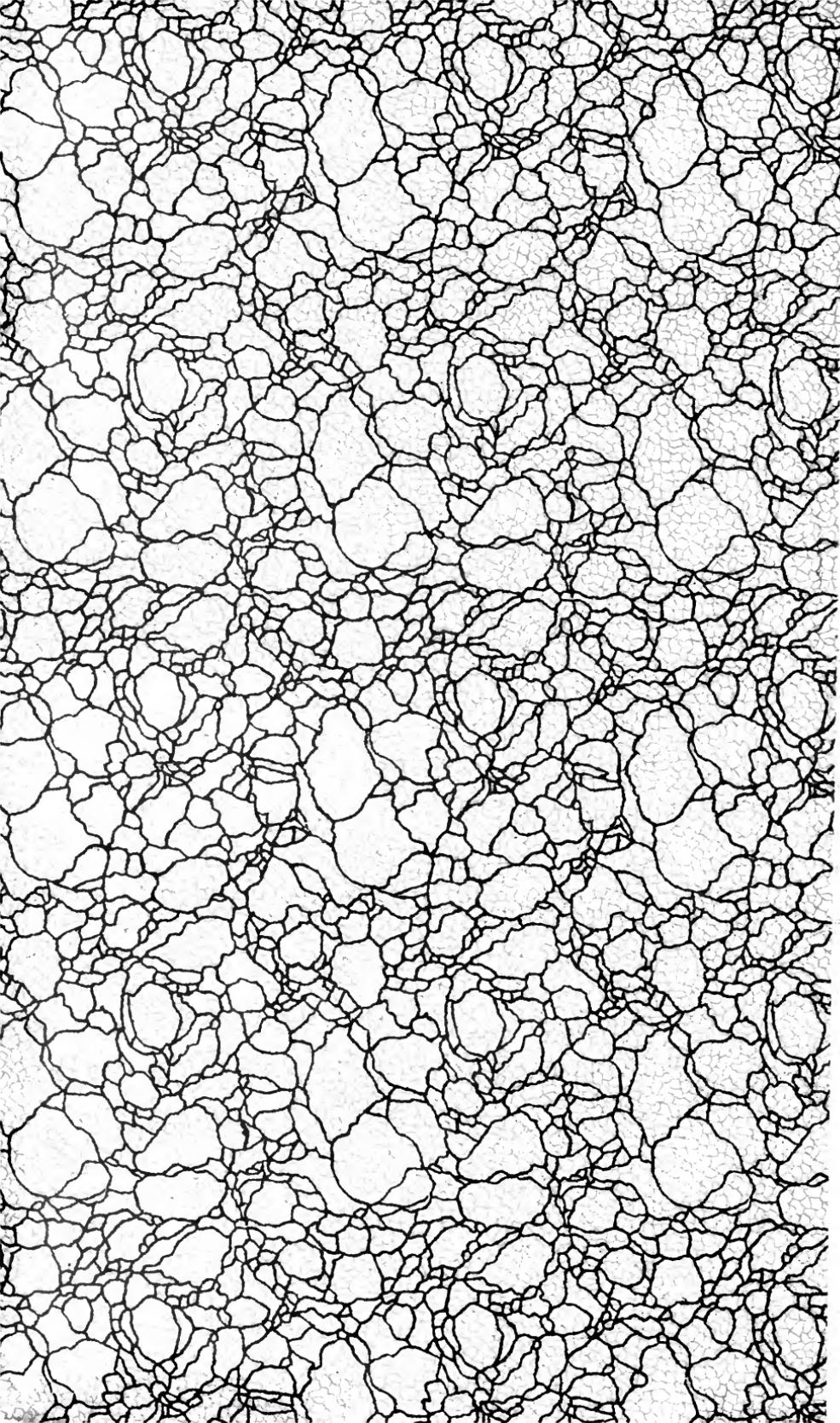
TABLE 3.—PHOSPHORUS, CALCIUM, AND NITROGEN REQUIRED BY CROPS, COMPARED WITH THAT POSSIBLE OF SOLUTION WHEN NITRITE BACTERIA ACT UPON TRI-CALCIUM PHOSPHATE

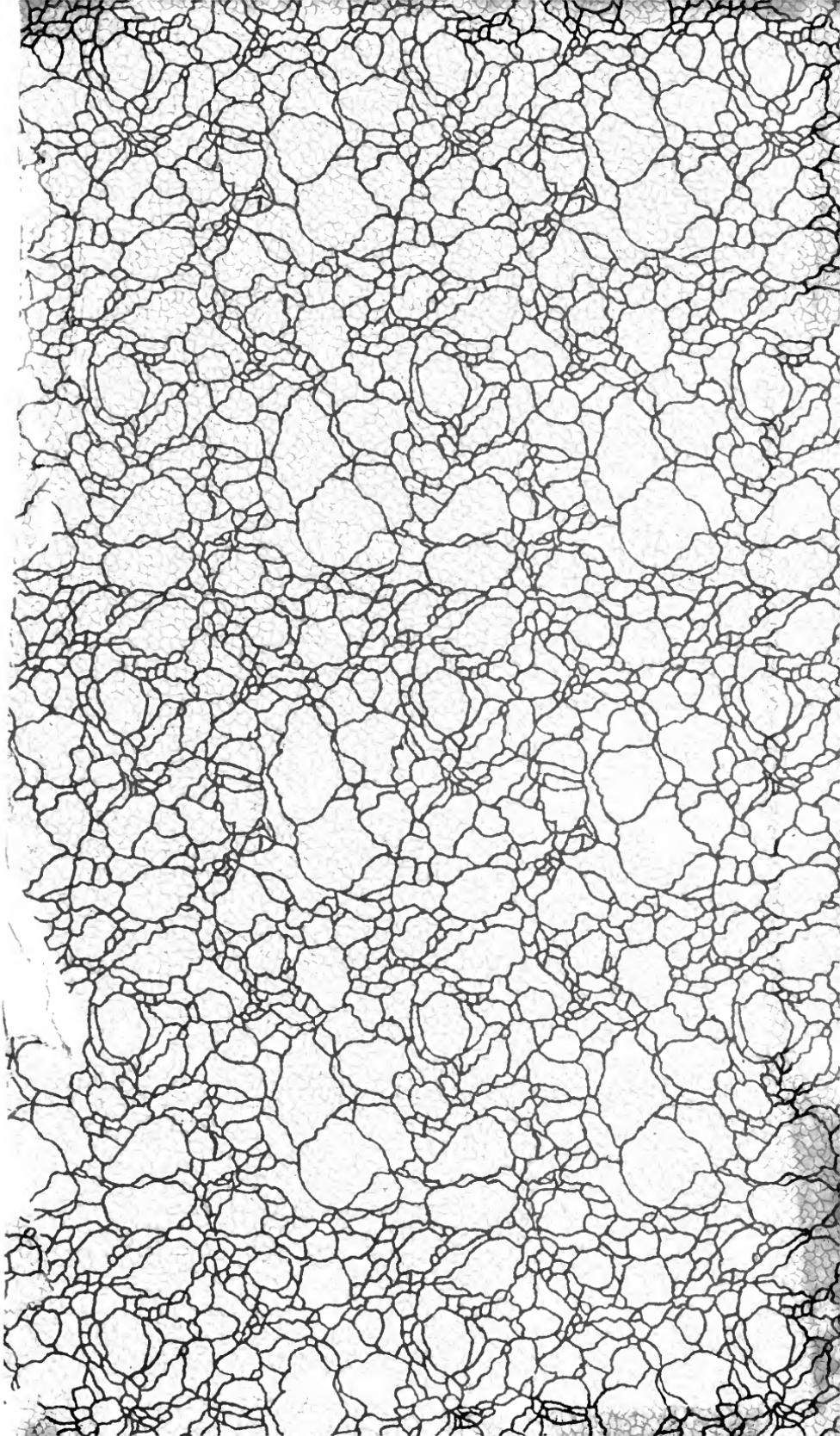
(Expressed in pounds)

Crop	Nitrogen	Phosphorus		Calcium	
	Required	Required	Possible	Required	Possible
Corn					
Grain, 100 bu.					
Stover, 3 tons....					
Cobs, $\frac{1}{2}$ ton.....	150	23	166	22	321
Wheat					
Grain, 50 bu.					
Straw, $2\frac{1}{2}$ tons.....	96	16	107	11	206
Oats					
Grain, 100 bu.					
Straw, $2\frac{1}{2}$ tons.....	97	16	108	17	208
Timothy, 3 tons.....	76	9	84	20	163

The figures show that there is possible of solution from this biochemical process about 7 times as much phosphorus as corn, wheat, or oats require, and 9 times as much as timothy requires. Greater differences occur in the calcium figures, there being possible of solution 14 times that required for corn, 18 times that required for wheat, 12 times that required for oats, and 8 times that required for timothy. It is evident that nitrite bacteria find in pure rock phosphate a highly satisfactory source of phosphorus.

The question, Will the calcium of raw rock phosphate suffice to neutralize the acid produced by nitrite bacteria; and, if so, will phosphorus be made soluble? has been answered in the affirmative from the results of this work.





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